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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Kamm *et al.*	Examiner:	Yu, J. R.
Serial No.:	09/815,528	Art Unit:	3764
Filed:	March 23, 2001		
Title:	METHOD AND APPARATUS FOR STIMULATING ANGIOGENESIS AND WOUND HEALING BY USE OF EXTERNAL COMPRESSION		

Assistant Commissioner for Patents
Washington, DC 20231

Sir:

DECLARATION UNDER 37 C.F.R. § 1.132

I, Roger D. Kamm, declare as follows:

1. I am an inventor of the subject matter disclosed and claimed in United States patent application Serial Number 09/815,528, filed March 23, 2001, and entitled "METHOD AND APPARATUS FOR STIMULATING ANGIOGENESIS AND WOUND HEALING BY USE OF EXTERNAL COMPRESSION".

2. I am a Professor in the Department of Mechanical Engineering and the Biological Engineering Division at the Massachusetts Institute of Technology, Cambridge, Massachusetts, one of the assignees of the above-referenced patent application. My research has focused on biomedical fluid dynamics, computational fluid dynamics, cell mechanics, and biomedical engineering. I have published numerous peer reviewed articles in this field and have made numerous technical presentations at professional meetings. A copy of my curriculum vitae is attached hereto as **Exhibit A**.

3. I have read the Office Action mailed October 9, 2002, and understand that the Examiner requests further evidence regarding the invention as claimed in the present application and

specifically as it pertains to the patentability of the claimed invention with respect to its operability and utility in stimulating angiogenesis and wound healing.

4. Shear stress is considered to be one of the major stimulatory factors mediating angiogenesis. This stimulatory effect is believed to be due to activation of the endothelium, which leads directly or indirectly to changes in expression of various cell adhesion molecules and growth factors. These factors provide the necessary environment for new blood vessel growth. For example, shear stress levels of 10-25 dynes/cm^2 have been shown to result in increased expression of monocyte chemoattractant protein-1 (MCP-1) and granulocyte-monocyte colony-stimulating factor (GM-CSF) in human umbilical vein endothelial cells (HUVEC) relative to static controls (Shyy *et al.* "Fluid shear stress induces a biphasic response of human monocyte chemotactic protein 1 gene expression in vascular endothelium" *Proc. Natl. Acad. Sci. USA* 91:4678-4682, 1994; Kosaki *et al.* "Fluid shear stress increases the production of granulocyte-macrophage colony stimulating factor by endothelial cells via mRNA stabilization" *Circ. Res.* 82:794-802, 1998).

5. Further studies have been conducted under my supervision to investigate the effect of shear stress on the expression of angiogenesis-related genes, including vascular endothelial growth factor (VEGF), MCP-1, GM-CSF, Angiopoietin 1 (Ang 1), and Ang 1 receptor Tie2, in human endothelial cells. Microvascular cells (MVEC) and HUVEC cells were isolated by known procedures and cultured *in vitro*. Flow experiments were then performed by seeding cells overnight on fibronectin-coated inner surface silastic tubes of a shear flow chamber attached to a rotating device. After 2 hours of preconditioning at 0.14 dyne/cm^2, flow experiments in which the cells were subjected to low, medium, and high levels of shear stress (0.14, 10, and 65 dynes/cm^2) for 6 hours were performed. At the end of the experiments, the cells were collected, and their RNA was isolated and analyzed by Ribonuclease Protection Assay using radio-labeled riboprobes. A schematic of the experimental procedure is attached hereto as **Exhibit B**.

6. Medium levels of shear stress (10 dynes/cm^2) were found to significantly increase the expression of MCP-1 in both microvascular cells (MVEC) and human umbilical vein endothelial cells (HUVEC). See Figure 1 of **Exhibit C**.

7. Higher levels of shear stress (65 dynes/cm^2) resulted in increased expression of Angiopoietin-1 receptor Tie2 in HUVECs. However, no induction of Tie 2 was observed in MVECs. See Figure 2 of **Exhibit C**.

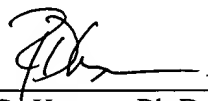
8. High levels of shear stress (65 dynes/cm^2) appear to cause a slight inhibition in VEGF and GM-CSF mRNA expression. Decreased levels of GM-CSF mRNA were also observed with intermediate levels of shear stress (10 dynes/cm^2). See Figures 3 and 4 of **Exhibit C**.

9. Our data indicate that the expression of genes known to influence angiogenesis is affected by shear stress. Increased levels of shear stress resulted in the increased production of MCP-1 and Tie2, while VEGF and GM-CSF seem to be decreased. Given these results *in vitro*, it is reasonable to assume that similar results could be achieved *in vivo* using the methods claimed in the present application to induce changes in the shear stress experienced by the endothelial cells of the subject. The Example in the Specification offers a mathematical model of the human vasculature to show that this increased shear stress can be accomplished using graded sequential compression. Therefore, the Specification and the results described herein support the utility and operability of the claimed invention.

10. In addition to these *in vitro* studies performed under my direction, several published clinical studies by others have suggested that increased flow and shear stress may promote angiogenesis. For example, Delis *et al.* have demonstrated that intermittent external compression applied to lower extremities can enhance collateral circulation in patients with peripheral vascular disease (Delis *et al.* "Improving walking ability and ankle brachial pressure indices in symptomatic peripheral vascular disease with intermittent pneumatic foot compression: A prospective controlled study with one-year followup" *J. Vas. Res.* 31:650-661, 2000; Delis *et al.* "Effects of intermittent pneumatic compression of the calf and thigh on arterial

calf inflow: a study of normals, claudicants, and grafted arteriopaths" *Surgery* 129(2):188-195, 2001). Others have shown that intermittent external compression applied to lower extremities can lead to the development of collateral circulation and improved myocardial perfusion in patients with chronic coronary artery disease (Soran *et al.* "Enhanced external counterpulsation in the management of patients with cardiovascular disease" *Clinical Cardiology* 22:173-178, 1999; Arora *et al.* "The Multicenter Study of Enhanced External Counterpulsation (MUST-EECP): Effect of EECP on Exercise-Induced Myocardial Ischemia and Anginal Episodes" *J. Am. College Card.* 33:1833-1840, 1990). In addition, enhanced external counter pulsation has been shown to lead to dramatically increased coronary re-perfusion pressure and flow following treatment (Michaels *et al.* "Left ventricular systolic unloading and augmentation of intracoronary pressure and Doppler flow during enhanced external counterpulsation" *Circulation* 106(10):1237-1242, 2002).

11. I, Roger D. Kamm, declare that all statements made herein of my own knowledge are true and that these statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful, false statements and the like are made punishable by fine or imprisonment, or both, under § 1001 of Title 18 of the United States Code and that such willful, false statements may jeopardize the validity of the application or any patents that may issue thereon.



Roger D. Kamm, Ph.D.

2/8/03

Date